A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia

TRIAL Relapsed AML 2001/01

To be performed within the International BFM Study Group (I-BFM-SG) and in collaboration with the UK MRC Childhood Leukaemia Working Party, the St. Jude Children's Research Hospital, the NOPHO, the FRALLE/LAME, SHOP and several other groups

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The writing committee has made every effort to avoid errors in this treatment protocol. However, the committee nor the study coordinators take responsibility for any errors that still may occur. It is the responsibility of the individual colleagues who use this protocol and its guidelines, to do so appropriately. In view of the complex international character of the study participants, there is no central patient insurance. Each group or center has to take care of that, if necessary.
A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia

**TRIAL Relapsed AML 2001/01**

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### Abbreviations

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<th>Definition</th>
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<tr>
<td>AML</td>
<td>acute myeloid leukemia</td>
</tr>
<tr>
<td>ara-CTP</td>
<td>arabinosylcytosine 5’-triphosphate</td>
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<td>CR</td>
<td>complete remission</td>
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<td>DNX</td>
<td>DaunoXome®</td>
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<td>FAB</td>
<td>French-American-British classification</td>
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<td>FLAG</td>
<td>fludarabine, cytarabine and G-CSF</td>
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<td>FS</td>
<td>fractional shortening</td>
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<td>HID</td>
<td>haplo-identical donor</td>
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<tr>
<td>ITH</td>
<td>intrathecally</td>
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<td>IV</td>
<td>intravenously</td>
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<td>LP</td>
<td>lumbar puncture</td>
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<td>LV</td>
<td>left ventricle</td>
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<tr>
<td>LVET</td>
<td>left ventricular ejection time</td>
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<td>MRD</td>
<td>minimal residual disease</td>
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<tr>
<td>MSD</td>
<td>matched sibling donor</td>
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<tr>
<td>MUD</td>
<td>matched unrelated donor</td>
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<tr>
<td>NR</td>
<td>non response</td>
</tr>
<tr>
<td>PR</td>
<td>partial response</td>
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<tr>
<td>SC</td>
<td>subcutaneously</td>
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<td>SCT</td>
<td>stem cell transplantation</td>
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<tr>
<td>SF</td>
<td>see FS</td>
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<tr>
<td>UNL</td>
<td>upper normal level</td>
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<tr>
<td>VP-16</td>
<td>etoposide</td>
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Summary

Relapsed and refractory acute myeloid leukemia (AML) in children is a rare problem, but has a poor prognosis. We therefore designed an international multicenter open label randomised phase III trial in children with such a disease. Reinduction treatment will be done with 2 courses of combination chemotherapy, with FLAG (fludarabine, ara-C and G-CSF) in both courses as standard treatment. In the first course there will be a randomisation for liposomal daunorubicin (DaunoXome®) to be added or not. The second course should always concern FLAG. If patients have >20% of blasts in the bone marrow after the 1st course, or if they are not in complete remission (CR) after the 2nd course, they will go off protocol. Patients in CR after reinduction treatment can immediately proceed to stem cell transplantation. Consolidation chemotherapy should be given if SCT is delayed. A 3rd course of intensive chemotherapy (VP16 and continuous infusion with cytarabine) is the general recommendation. In selected patients, a low intensity consolidation may be preferred, and such a schedule is described as well. The type of SCT is based on the risk-group. Preferably, a matched sibling donor (MSD) SCT is performed. If a MSD is not available all patients are candidates for a matched unrelated donor (MUD) SCT. If a MUD is also not available, patients with primary refractory disease, early relapse (within 1 year from diagnosis), or ≥2nd relapse, are candidates for the more experimental haplo-identical donor (HID) SCT in view of the dismal prognosis. However, patients with a late relapse (>1 year from initial diagnosis) have a better prognosis and should be offered an autologous SCT if a MSD or MUD SCT is not possible. Only in case of autologous SCT, maintenance treatment and/or adjuvant immunotherapy could be considered.

Main objectives of the study are to determine the efficacy and toxicity of DaunoXome® when added to FLAG in children with relapsed and refractory AML. In addition, the study will prospectively determine the clinical outcome of these patients, stratified according to the different risk groups (refractory disease, early relapse, late relapse, multiple relapse). Additional objectives are to determine the clinical relevance of minimal residual measurements, in vitro cellular drug resistance data, cell biological and molecular features and pharmacokinetic data of DaunoXome®, in these patients.

The study expects to accrue up to 100 patients annually, and will run about 4 years.
Main objectives

Primary
1. Determine the efficacy of liposomal daunorubicin (DaunoXome®) when added to FLAG in the 1st course as compared to patients treated with FLAG only

Secondary
1. Determine the toxicity of DaunoXome® when added to FLAG, in terms of mucosal toxicity, bone marrow aplasia, short- and long-term cardiotoxicity and other side-effects, as compared to patients treated with FLAG only
2. Determine the long-term clinical outcome prospectively in a large group of children with refractory and relapsed acute myeloid leukemia

Additional objectives (add-on research studies)

1. Determine the changes in minimal residual disease over time, and the prognostic significance of minimal residual disease determined at various time-points
2. Determine the relation between in vitro cellular drug resistance and clinical and cell biological features, minimal residual disease and clinical outcome in this patient-group
3. Determine the pharmacokinetics of DaunoXome® in relation to its toxicity and efficacy
Background and introduction

Newly diagnosed acute myeloid leukemia (AML) is a rare disease in children. The prognosis has improved using intensive chemotherapy with or without stem cell transplantation. However, 5-10% of patients do not achieve first complete remission (CR) due to resistant disease (primary refractory AML), and 30-50% of CR patients relapse. Primary refractory AML and relapsed AML have a poor prognosis, with an overall survival of less than 25%. For first relapse disease, the prognosis mainly depends on the time of relapse. If early, defined as within 1-1.5 years from initial diagnosis, the second CR rate is about 50%, and overall survival 10% or less. If late, defined as after 1-1.5 years from diagnosis, the second CR rate is 80-90% and the overall survival up to 40%. Multiple relapsed AML has an even worse prognosis. Several treatment schedules have been studied recently, and progress seems feasible using new drugs and new drug combinations. The currently available alternatives are described below.

Liposomal daunorubicin (DaunoXome®)

Daunorubicin is a well-known anthracycline with proven efficacy in AML. Anthracyclines are intensely used in first-line treatment, which limits its use in case of refractory or relapsed disease, because of its cardiotoxicity. This cardiotoxicity is dose-related, and becomes an increasing problem in case of higher cumulative doses (Lipshultz 1991). Such cumulative doses have normally been given in first-line treatment. Another cumbersome side-effect of anthracyclines is mucositis. DaunoXome® is a combination of daunorubicin and a unilamellar liposomal transport system. Because of the chemical formula, the compound is relatively stable in the plasma. Because of the preferential release of free daunorubicin intracellularly, the plasma level of free daunorubicin is lower than in case of infusion with daunorubicin itself. In a mouse model, liposomal doxorubicin had no cardiotoxicity like in saline-treated controls, but did have significant antileukemic activity (Forssen 1981). In an isolated perfused rat model, DaunoXome® had no cardiotoxicity in contrast to free daunorubicin (Pouna 1996). DaunoXome® had a relatively low incorporation in heart muscle as compared to tumor cells (Forssen 1992). The above may explain the absence of cardiotoxicity of DaunoXome® in 277 AIDS patients with Kaposi’s sarcoma, who received cumulative doses of DaunoXome® of up to 1700 mg/m², >600 mg/m² in 53 patients at a total group mean cumulative dose of 481 mg/m² (Gill 1996). Out of 979 Kaposi’s sarcoma patients treated with DaunoXome®, 1 patient developed cardiotoxicity with clinical symptoms (Summary of Product Characteristics, Gilead Sciences, 5 June 2001). In general, liposomal anthracyclines cause less cardiotoxicity at higher cumulative doses than conventional anthracyclines (Levitt 1999). Animal solid tumor studies showed that at equivalent daunorubicin dosages, DaunoXome® had significantly increased antitumor activity and less toxicity as compared to free daunorubicin (Corbett 1978, Forssen 1994). Significant penetration of DaunoXome® into the cerebrospinal fluid and brain is unlikely, since liposomes cross the blood-brain barrier poorly, if at all (Tökés 1980). This is also the case for free anthracyclines. However, the penetration may be different in e.g. brain tumors (Zucchetti 1999). A clinical study of the AML-BFM group (Creutzig et al., unpublished data) is in progress, in which DaunoXome® is given in combination with cytarabine in relapsed AML patients. Initially, DaunoXome® at 60 mg/m²/day was given on days 1 and 5 of a course including 4 days of continuously iv cytarabine (500 mg/m²/day for 4 days), which course was repeated after 4-5 weeks. Because of limited toxicity, the current cohort of patients is being treated with an extra infusion of DaunoXome® on day 3 of each of the 2
courses. No cardiotoxicity has been seen so far, but follow-up is short. Based on these data, it was decided to include DaunoXome® in this treatment protocol.

**FLAG +/- Idarubicin**
The favorable effect of combining fludarabine and cytarabine, leading to an increased arabinosylcytosine 5′-triphosphate (ara-CTP) accumulation as compared to that achieved by cytarabine alone, was initially described by Gandhi et al. (1993). In addition, it has been reported that G-CSF combined with cytarabine may render AML cells more sensitive to the latter drug (Tafuri 1990), which may be explained by the increased ara-CTP accumulation - associated with increased deoxycytidine kinase activity – in case of pre-exposure to G-CSF as reported by Braess et al. (2000). Finally, Gandhi et al. (1995) reported that G-CSF significantly increased the accumulation of the active metabolite of fludarabine. Thus, the combination of fludarabine, cytarabine and G-CSF (FLAG) emerged, although the role of G-CSF has been questioned (Estey 1994). Several colleagues in the UK used FLAG for reinduction of relapsed AML. Retrospectively, data on 40 patients were obtained using a questionnaire (Gibson et al., unpublished data), with subsequent CR in 21/30 (70%) relapse patients and CR in 3/7 primary refractory patients. One patient died of pneumocystis carinii pneumonia. Grade 4 hematological toxicity was the rule. Fleischhack et al. (1998) treated 38 (update: 44) relapsed AML patients with IDA-FLAG, ie, idarubicin added to FLAG, followed by again IDA-FLAG or FLAG. Three patients received FLAG only. Toxicity was marked. Numbers were small, but the time of myelosuppression was shorter after FLAG than after IDA-FLAG. Except for grade 4 hematological toxicity, infections with often pulmonary involvement was a relatively frequent complication, and were more often seen after IDA-FLAG than after FLAG. CR rates were 56% and 75% in early and late relapses respectively (updated but not published data: 58% and 78% respectively). This protocol applies the FLAG schedule with G-CSF starting 1 day before and to be continued during chemotherapy, based on the above summarised studies.

**FLAG + DaunoXome®**
Fleischhack et al. (Bonn, unpublished data) have piloted this regimen in 10 patients with relapsed AML. Toxicity was tolerable and similar as with IDA-FLAG. Five patients had mild mucositis, eight fever of unknown origin, one gram-positive bacteremia, and one a pneumonia. Five patients achieved CR and proceeded to SCT, two patients were partial responders and three patients did not respond and died. This pilot study shows that FLAG in combination with DaunoXome® is feasible and has antileukemic activity. However, 2 consecutive courses of FLAG + DaunoXome® (3 x 60 mg/m²/day) is anticipated to be too toxic.

**Other reinduction chemotherapy regimens**
Cytarabine or other agents have been studied in combination with well-known drugs such as etoposide and amsacrine, with significant antileukemic activity (Miller 1991, Ozkaynak 1998, Steuber 1996, Whitlock 1997, Baruchel unpublished). Data on the use of new agents such as topotecan in children have not been reported yet. Cladribine® (2-chloro-deoxyadenosine) has been studied in relapsed AML as well. The response rate in 17 children was 59%, including a CR rate of 47% (Santana 1992). However, these investigators subsequently investigated this drug in 93 children with newly diagnosed AML or myelodysplastic syndrome (MDS), and found that at an overall CR rate of 40% after 2 courses of cladribine®, patients with FAB type M5 responded significantly better
than non-M5 patients, with CR rates of 71% and 24% respectively. Therefore, cladribine does not seem very active in non-FAB M5 type cases.

**CNS prophylaxis and treatment**

Overt CNS leukemia is relatively rare in AML. This protocol applies high-dose chemotherapy, with an anticipated CNS prophylactic effect as well. This is also true for the conditioning regimen (see later), that all children are supposed to get. There is virtually no literature available on CNS prophylaxis and treatment in this patient-group to further refine or substantiate the recommendations given in this protocol, which were therefore extensively discussed and agreed upon.

**Consolidation chemotherapy**

To consolidate an achieved CR, and to have time to prepare a transplant if not available immediately, further chemotherapy seems indicated. Whether this is really necessary and if so, which drugs should be used, is essentially unknown. Yet, in these high-risk patients, further intensive treatment seems justified. Therefore, a schedule of VP16 and cytarabine given by continuous infusion has emerged. In case of a delayed SCT and a contra-indication for this high-intensity consolidation regimen, a low-intensive combination of orally thioguanine and cytarabine subcutaneously is proposed. Extensive experience with this regimen has been obtained within the BFM-AML protocols.

**Stem cell transplantation (SCT)**

Allogeneic SCT is associated with major morbidity and mortality. Therefore, allogeneic SCT should be considered in the context of the prognosis with chemotherapy only. In patients with primary refractory and relapsed AML, this prognosis is very poor. Moreover, few long-term survivors have been described after chemotherapy only for this particular disease, in contrast to chemotherapy followed by SCT. Although not proven in properly designed randomised clinical trials, allogeneic SCT may have a bigger impact on the prognosis than autologous SCT, because: 1) a graft-versus-leukemia effect may be present in the allogeneic, but not (or less) in the autologous setting, and 2) the autologous transplant is likely to contain minimal residual leukemia cells, which may cause a relapse. On the other hand, allogeneic SCT may be complicated by graft-versus-host-disease. We do recommend allogeneic SCT for all patients who achieve CR, preferably using a matched sibling donor (MSD), or if not available, a matched unrelated (MUD) donor. Patients at higher risk of relapse, i.e. primary refractory disease, early relapse, and ≥2nd relapse, are eligible for the more experimental haplo-identical donor (HID) SCT if both a MSD and a MUD are lacking. For patients with a late relapse such treatment is too experimental in view of their better prognosis, and autologous SCT may be used instead. There are several proposals for the conditioning regimen, inclunding a combination of busulfan, cyclophosphamide and melphalan. Experiences using this regimen in children with myelodysplastic syndrome were good (Locatelli 1994). Depending on the type of donor, additional immunosuppression may be required in conditioning. Patients who were not irradiated previously and who will have a SCT for the 1st time should have for instance TBI/cyclophosphamide (also depending on age) as conditioning instead of busulfan, cyclophosphamide and melphalan.

**Salvage treatment**

A significant proportion of the protocol patients will not respond to the 1st course of reinduction chemotherapy or will not achieve complete remission after 2 courses. These patients, with a very poor prognosis, did not benefit from conventional chemotherapy and
will go off this study. Recent experiences with Mylotarg® (CMA-676, anti-CD33 linked to calicheamicin) in adult AML are relatively favorable in a similar group of patients, with a 30-40% complete response rate (Sievers 2000, 2001). Therefore, this type of targeted chemotherapy with Mylotarg® can be offered to these off-study patients (as well as to patients who relapse after initially having achieved CR on protocol Relapsed AML 2001/01) in the setting of the separate phase II study Relapsed AML 2001/02. That treatment protocol is available from the international study coordinator.

**Conclusion**

The above summary shows that the FLAG regimen seems relatively effective with tolerable toxicity. It is essentially unknown if the addition of an anthracycline would improve the antileukemic activity of FLAG. Another point of concern is the cardiotoxicity of anthracyclines, which is a major potential problem in these patients because of their initial treatment leading to high cumulative doses of these drugs already. However, anthracyclines are among the most effective drugs in AML. Therefore, it appears that a randomisation between FLAG and FLAG + DaunoXome® is a good study question, including the most effective regimen currently known.

The prognosis of refractory and relapsed AML is very poor. Thus, there is a clear need to improve the prognosis, but this can only be achieved in the setting of large intergroup trials because of the rarity of the disease. This protocol describes such an international randomised phase III study which aims at improving the outcome of these children and to learn more about the cause(s) of the poor treatment response. The obtained knowledge may also be useful for the future treatment of newly diagnosed AML, especially with respect to the use of DaunoXome® and FLAG.
Patient inclusion and exclusion criteria

Inclusion criteria
1. Children and adolescents <18 years of age at start of chemotherapy
2. Primary refractory AML
3. First relapsed AML
4. Patients with a second or subsequent relapsed AML that were not previously treated according to this particular protocol
5. Signed written informed consent according to local policies

Patients with a combined relapse, or an isolated extramedullary relapse, or a bone marow relapse (isolated or combined) with <20% blasts in the BM are eligible, also for randomisation. Although the primary end-point of this study can not be evaluated in patients with <20% blasts in the BM at he start of reinduction treatment, the secondary end-points can be evaluated.

Exclusion criteria
1. Symptomatic cardiac dysfunction (CTC grade 3 or 4) and/or a Fractional Shortening at echocardiography below 29%
2. A Karnofsky performance status <40% (children >=16 years) or an Lanksy performance status of <40% (children < 16 years) before start of chemotherapy
3. Any other organ dysfunction (CTC grade 4) that will interfere with the administration of the therapy according to this protocol (e.g. transaminases >10 times the UNL)
4. Inability to potentially complete the treatment protocol for any other reason
5. FAB type M3 (please refer to your local group for one of the treatment alternatives)

Please do register all patients with relapsed or refractory AML, independent of treatment according to this protocol, so that selection bias may be considered.
Chemotherapy schedules

Reinduction - general information

Appendix L shows the outlines of this treatment protocol. Before the start of treatment, each patient must be randomised for the addition of DaunoXome® or not to FLAG. The procedure for registration and randomisation is explained on page 34 and in Appendix B. Please also register all patients who are not randomised for whatever reason. The second course will consist of FLAG for all patients, except for non-responders (off-study). Patients not in CR after the 2nd reinduction course are also off-study. After the 2nd course, one can proceed immediately to SCT, if available. If not, we recommend an intensive consolidation with VP16 and continuous infusion cytarabine. In selected patients (e.g., concerns of cumulative toxicity) a low-intensive consolidation with thioguanine orally and cytarabine subcutaneously may be preferred. SCT is recommended for all patients who achieved CR, ideally using a MSD or a MUD. If not available, the use of a haplo-identical donor is proposed to be restricted to patients with a very high risk of relapse (see later), while autologous SCT is recommended for patients with late relapsed AML.

Other considerations:
- Send unstained slides of BM and PB to your reference laboratory for central diagnosis, and if possible send material for research studies in order to be able to address the additional objectives of the study (see information on page 36 and the appendices).

- Determine the BM leukemic blast count on day +15 after the start of the 1st course, as well as the time required to clear the PB of leukemic blasts, if any. The purpose is to monitor early clinical response, which may have prognostic significance. Analyses should therefore be done centrally by each group (if no central laboratory, this can also be done in Münster, Germany).

- The 2nd course of treatment should start not earlier than 28 days after the start of the 1st course. The 2nd course may start once the neutrophils are ≥1.0x10⁹/l and the platelets are >50x10⁹/l (without transfusions) and if the clinical condition allows it. A BMA should be done (and slides sent to your reference laboratory!) not earlier than 28 days after the start of the 1st course, before the start of the 2nd course. However, if the start of the 2nd course is delayed, a BMA should be done not later than 42 days after the start of the 1st course. If the patient does not qualify for the start of the 2nd reinduction course because of too low neutrophils and/or platelets, which is likely to be caused by residual leukemia in the bone marrow (with a partial remission recorded), then treatment should be continued.

- Please be aware of the follow-up guidelines (page 28 and further), including cardiotoxicity monitoring (page 30).
Special measures for reinduction

High cell counts
Patients with high peripheral blast counts (>50-100x10^9/l) and significant organomegaly have increased problems related to metabolic abnormalities, bleeding, and hyperviscosity. Platelets should be transfused if < 15-30x10^9/l; in this phase, hemoglobin should not be raised above 6 mmol/l (= 9.6 g/dl); treatment of coagulation disorders is required. The use of leukopheresis, exchange transfusion, or hemodialysis may be necessary. To prevent tumor lysis syndrome, the patient should be well hydrated, alkalinized and get allopurinol before start of therapy:
- Allopurinol: 200-300 mg/m2 p.o. divided q 8-12 hours; give at least 2 doses prior to initiation of therapy (alternatively, uricozyme may be used)
- Hydration: 2400-3000 ml/m2/day; be careful with potassium containing solutions; electrolyte substitutions only based on laboratory results; check fluid balance
- NaHCO3: 4 g/m2/day (= 50 mEq/m2/day) to maintain urine pH 7-8

High dose ARA-C
During cytarabine and for 24-48 hours after completion, dexamethasone ophthalmic solution or isotears (q 6 hours) should be used to prevent cytarabine induced conjunctivitis.
Because of the association of streptococcus viridans infection and high dose cytarabine, penicillin prophylaxis is recommended from completion of cytarabine administration until neutrophil recovery.
Nurses and doctors should be aware of the relatively high (up to 15%) incidence of neurotoxicity, which may necessitate to interrupt or stop high-dose cytarabine administration.
Pyridoxine (300 mg/m2/day in 2 doses) might ameliorate cytarabine-induced mucositis.

Granulocyte colony stimulating factor
G-CSF will be used as part of the FLAG regimen (please refer to “background and introduction”). After discontinuation at day 6 it is recommended to restart G-CSF at day 15 of each reinduction course in order to shorten the period of neutropenia in these patients who all are at high risk for severe infections, of which the occurrence is correlated with the duration of neutropenia. G-CSF can be stopped again upon neutrophil recovery, as locally defined. The use of G-CSF for prevention or treatment of chemotherapy induced fever and neutropenia before day 15 is considered useless.

Irradiation of blood products
Mainly due to fludarabine and body irradiation, profound and long term lymphocytopenia can be expected to occur. Therefore, cellular blood products should be irradiated with 25 Gy to prevent transfusion related graft versus host disease, at least up to 6 months after the last fludarabine administration. Of course, all blood products should be leukocyte-depleted as well. Similarly, irradiation and leukocyte-depletion of the blood products is required at least up to 6 months after SCT.
REINDUCTION FLAG

randomise to know if FLAG or FLAG + DaunoXome® should be given as 1st course!

Perform BMA and LP first! Please note: BMA day 15 after the 1st course, and record day of peripheral blood blast clearance!

BMA after course 1 not earlier than 28 days after the start of course 1, but in case of delay, not later than day 42 after the start of course 1.

Course 2 (always FLAG) may start not earlier than 28 days after the start of course 1, and only in case of good clinical condition, platelets > 50x10⁹/l (without transfusions) and neutrophils > 1.0x10⁹/l. An exception are patients with partial remission after course 1, who retain too low neutrophils and/or platelets, likely to be due to residual leukemia. These patients – if in good enough clinical condition – may continue treatment irrespective of the blood counts, after BMA.

Fludarabine 30 mg/m²/day | | | | | | 30 minutes i.v. infusion
Cytarabine 2000 mg/m²/day | | | | | 3 hours i.v. infusion (begin 4 hours after start of Fludarabine)
GCSF 200 µg/m²/dose | | | | | | | | | | 0 1 2 3 4 5 days
Subcut. or i.v. (for 6 days, given before fludarabine; restart on day 15 until neutrophil recovery)
Intrathecal medication (see CNS prophylaxis and treatment)

The second course of FLAG is similar to the 1st course (if randomised to receive FLAG in course 1)

In case of a body weight <12 kg, the prescribed doses should be corrected as follows: [weight (kg) x dose (per m²)] divided by 30. This should be considered at each treatment course.
REINDUCTION FLAG + DaunoXome®

randomise to know if FLAG or FLAG + DaunoXome® should be given as 1st course! (2nd course always FLAG)

Perform BMA and LP first! Please note: BMA day 15, and record day of peripheral blood blast clearance!

BMA after course 1 not earlier than 28 days after the start of course 1, but in case of delay, not later than day 42 after the start of course 1.

Fludarabine 30 mg/m²/day 30 minutes i.v. infusion

Cytarabine 2000 mg/m²/day 3 hours i.v. infusion (begin 4 hours after start of Fludarabine)

GCSF 200 µg/m²/dose Subcut. or i.v. (for 6 days, given before fludarabine; restart on day 15 until neutrophil recovery)

DaunoXome® 60 mg/m²/day 60 min. i.v. infusion, after fludarabine

Intrathecal medication (see CNS prophylaxis and treatment)

In case of a body weight <12 kg, the prescribed doses should be corrected as follows: [weight (kg) x dose (per m²)] divided by 30. This should be considered at each treatment course.
CONSOLIDATION - high intensity

Perform BMA + LP first! BMA after the 2nd reinduction course not earlier than 28 days after the start of course 2, but in case of delay, not later than day 42 after that start. If no CR after course 2: off study

To be started not earlier than 28 days after the start of course 2, and only in case of good clinical condition, platelets > 50x10⁹/l (without transfusion) and neutrophils > 1.0x10⁹/l

To be given as consolidation to all patients, if a SCT is not available immediately.

Ara-C 500 mg/m²/day cont. infusion for 4 days

VP-16 100 mg/m²/dose every 12 hours in 1 hour infusions

Intrathecal medication (see CNS prophylaxis and treatment)

In case of a body weight <12 kg, the prescribed doses should be corrected as follows: [weight (kg) x dose (per m²)] divided by 30. This should be considered at each treatment course.
CONSOLIDATION - low intensity

Perform BMA and LP first! BMA after the 2\textsuperscript{nd} reinduction course not earlier than 28 days after the start of course 2, but in case of delay, not later than day 42 after that start. If no CR after course 2: off study

To be started not earlier than 28 days after the start of course 2, and only in case of good clinical condition, platelets > 50x10\textsuperscript{9}/l (without transfusion) and neutrophils > 1.0x10\textsuperscript{9}/l

To be given only if a SCT is not available immediately, to patients who will not tolerate the 3\textsuperscript{rd} course of high-intensity consolidation chemotherapy.

Thioguanine 100 mg/m\textsuperscript{2}/day
Max. 4 weeks 1 daily oral dose (evenings)

Cytarabine 75 mg/m\textsuperscript{2}/day
1 daily subcutaneous injection
days 1-4 and 15-18

Intrathecal medication
(see CNS prophylaxis and treatment)

In case of a body weight <12 kg, the prescribed doses should be corrected as follows: [weight (kg) x dose (per m\textsuperscript{2})] divided by 30. This should be considered at each treatment course.

**Criteria for reduction or discontinuation of consolidation therapy:**

<table>
<thead>
<tr>
<th></th>
<th>Leukocytes</th>
<th>% Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6-Thioguanin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>&gt; 3.0x10\textsuperscript{9}/l</td>
<td>150</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>&gt; 2.0x10\textsuperscript{9}/l; &lt; 3.0x10\textsuperscript{9}/l</td>
<td>100</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>&gt; 1.0x10\textsuperscript{9}/l; &lt; 2.0x10\textsuperscript{9}/l</td>
<td>50</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>&lt; 1.0x10\textsuperscript{9}/l</td>
<td>0</td>
</tr>
<tr>
<td><strong>Cytarabine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>&gt; 2.0x10\textsuperscript{9}/l and Platelets</td>
<td>100</td>
</tr>
<tr>
<td>Platelets</td>
<td>&gt; 80x10\textsuperscript{9}/l</td>
<td>100</td>
</tr>
</tbody>
</table>

Below these counts discontinuation for 1 week
CNS PROPHYLAXIS AND TREATMENT

1. Prophylaxis:
There should be no evidence of CNS leukemia (see below). Cytarabine should be administered three times, intrathecally at age-adjusted doses (Table), via a lumbar puncture (LP). Give the first and second dose one day before the start of reinduction course 1 and 2 respectively (not simultaneously with high dose cytarabine). Give the third dose at the start of consolidation treatment. Cranial irradiation is not recommended.

<table>
<thead>
<tr>
<th>Age</th>
<th>Cytarabine, single dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 year</td>
<td>20 mg</td>
</tr>
<tr>
<td>1&lt;2 year</td>
<td>26 mg</td>
</tr>
<tr>
<td>2&lt;3 year</td>
<td>34 mg</td>
</tr>
<tr>
<td>≥ 3 year</td>
<td>40 mg</td>
</tr>
</tbody>
</table>

2. Treatment of CNS leukemia
CNS leukemia is defined on page 24. Triple intrathecal medication at age-adjusted doses (Table), by LP. The first dose should be given immediately before the start of reinduction course 1. The second and subsequent doses should be given every 7 days until 1 week after complete clearance of the CSF of leukemic blasts. Then, 2 more doses must be given, one immediately before the start of the second reinduction course, and the other at the start of consolidation treatment.

<table>
<thead>
<tr>
<th>Age</th>
<th>Cytarabine, every week</th>
<th>Methotrexate, every week</th>
<th>Prednisolone, every week</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year</td>
<td>16 mg</td>
<td>6 mg</td>
<td>4 mg</td>
</tr>
<tr>
<td>1&lt;2 year</td>
<td>20 mg</td>
<td>8 mg</td>
<td>6 mg</td>
</tr>
<tr>
<td>2&lt;3 year</td>
<td>26 mg</td>
<td>10 mg</td>
<td>8 mg</td>
</tr>
<tr>
<td>≥ 3 years</td>
<td>30 mg</td>
<td>12 mg</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

Cranial irradiation is not recommended, but may be considered in those patients who do achieve complete remission but who will not undergo SCT, and who did not receive cranial irradiation in the past.
**CONDITIONING REGIMEN - guidelines**

- To start if: CR, good clinical condition, platelets > 50x10^9/l. BMA and LP first!
- Allogeneic and autologous SCT: same regimen for BFM patients; other groups may apply different regimens, also depending on previous treatment (cranial irradiation, previous SCT). Additional immunosuppression according to local guidelines.

**BFM-patients**

<table>
<thead>
<tr>
<th>Day</th>
<th>Busulfan</th>
<th>Cyclophosphamide</th>
<th>Melphalan</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7</td>
<td>4 mg/kg/day, in 4 oral doses (if &lt; 3 years: 5 mg/kg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Use Mesna according to local policies

1. Alternatively i.v. busulfan (Busulfex®) may be considered, consult Prof. J. Boos (Munster, Germany)

Blood levels of busulfan should be monitored.

**CONDITIONING in not previously irradiated patients**: e.g. TBI/Cyclophosphamide

**Other groups**

<table>
<thead>
<tr>
<th>Day</th>
<th>Cyclophosphamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>-5</td>
<td>60 mg/kg/day</td>
</tr>
<tr>
<td>-4</td>
<td></td>
</tr>
<tr>
<td>-3</td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>-1</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Use Mesna according to local policies

**TBI**

- >10 year: 6 Gy
- 4-9 year: 7.5 Gy
- 2-3 year: 7 Gy
- <2 year: alternative regimen

**Stem cell reinfusion**

In case of a body weight <12 kg, the prescribed dose for melphalan should be corrected as follows: \[\text{weight (kg)} \times \text{dose (per m}^2\text{)}\] divided by 30.
STEM CELL TRANSPLANTATION (SCT)

Patients in complete remission after the 2 courses of reinduction chemotherapy, are eligible for SCT. Therefore, the search for a donor should start as soon as the diagnosis of refractory or relapsed AML has been made. The preferred type of SCT depends on the risk group and the donor availability.

I. Low risk = late (≥1 year from initial diagnosis) first relapse
   1) matched sibling donor SCT
   2) matched unrelated donor SCT
   3) autologous SCT

II. High risk = early (<1 year from initial diagnosis) first relapse, multiple relapse, refractory disease
   1) matched sibling donor SCT
   2) matched unrelated donor SCT
   3) haplo-identical donor SCT
   4) autologous SCT

Whatever risk group, one should in general avoid a SCT within 6 months after a previous allogeneic SCT in view of the toxicity of such procedures. If necessary, consolidation treatment according to the low intensity regimen could be given after the 3rd course of intensive consolidation chemotherapy to achieve this time period.

In case of an autologous SCT, stem cells that were obtained in stable first CR are preferred. If not available, stem cells should be harvested upon regeneration after the 2nd reinduction course. Peripheral blood stem cells are preferred, but bone marrow can be harvested as alternative. Also if stem cells are available from CR1, it is recommended to harvest stem cells again as back-up. Purging is not generally recommended. The goal is to harvest a total of ≥2.0 x 10^8 mononuclear cells/kg. The minimum number of CD34-positive cells required for reinfusion is 2.0 x 10^6/kg.

Because melphalan competes with phenylalanine for cellular uptake, iv nutrition including amino acids should not be given one day before until at least two days following melphalan.

Additional immunosuppression may be required, depending on the type of donor. Please apply the local guidelines.
OFF-STUDY PATIENTS

Patients who still have more than 20% leukemic blasts in the BM after the 1st course, or patients not in CR after the 2nd course of chemotherapy, should go off this study. Follow-up is still required, however. Similarly, patients who relapse on this treatment protocol are off study, but long-term clinical follow-up is urgently requested. Finally, patients who were not randomised, but who were eligible for this study or were diagnosed with relapsed or refractory AML, should be registered as well. Proper registration and follow-up of all of these and the study patients will enable the prospective study on the long-term clinical outcome of refractory and relapsed childhood AML. In addition, it will be important to determine whether there was a selection-bias for the patients entering this study.

Patients who fail on treatment protocol Relapsed AML 2001/01, and those who relapse despite achieving CR, are eligible for third line treatment with Mylotarg® (CMA-676, anti-CD33 linked to calicheamicin) in the setting of a separate I-BFM-SG phase II study, trial Relapsed AML 2001/02. That treatment protocol can be provided by the international study coordinator.
Drug information, expected toxicity and dose adjustments

Busulfan
This alkylating agent has somewhat unpredictable uptake after oral administration. Pharmacokinetic monitoring, if available, is recommended. The formula for intravenous use is an alternative. Main side-effects are: alopecia (rarely irreversible), nausea and vomiting, diarrhea, hyperpigmentation, growth retardation, myelosuppression, convulsions (consider antiepileptic drugs), lung fibrosis, and veno-occlusive disease.

Cyclophosphamide
This alkylating agent can cause nausea, vomiting, alopecia, myelosuppression, immunosuppression, hemorrhagic cystitis, stomatitis, SIADH, neurotoxicity, and lung fibrosis. Fertility may become impaired, especially if used at pubertal age. There seems to be a slightly increased risk of secondary malignancies. To prevent hemorrhagic cystitis, uromitexan (Mesna) should be used.

Cytarabine
This nucleoside analogue (pyrimidine antagonist) is an antimetabolite that is mainly metabolised in the liver. It may cause myelosuppression, hepatotoxicity, exanthema, malaise, and gastro-intestinal toxicity such as nausea, vomiting, stomatitis, and ileus. At high doses, fever, severe diarrhea, central nervous system disturbances such as somnolence, cerebellar ataxia and nystagmus, keratitis, veno-occlusive disease, pneumonitis and adult respiratory distress syndrome with streptococcus viridans infection are possible. Intrathecal administration has been associated with headache, fever, vomiting, and pleiocytosis, rarely with aseptic meningitis or central nervous system disturbances. To prevent keratitis, indifferent or corticoid eye drops every 4-6 hours are recommended during and until 12 hours after stopping cytarabine. Prolonged corticoid eye drops have side-effects as well, such as infection. Conjunctivitis should be treated with corticoid eye drops. To prevent streptococcal infection, penicilline prophylaxis may be considered starting as soon as neutropenia develops after high-dose cytarabine until neutrophils > 0.5x10⁹/l. Patients who developed grade 3 or 4 neurotoxicity following high-dose cytarabine should not receive further high-dose cytarabine, but are eligible for continuous lower-dose cytarabine.

Fludarabine
This nucleoside analogue (purine antagonist) is an antimetabolite that is excreted mainly in the urine. After phosphorylation, it is less susceptible to deamination than cytarabine. Main side-effects are myelosuppression, fever, chillis, malaise, nausea, and vomiting. More rare are auto-immune phenomena (hemolytic anemia), pneumonitis, and CNS symptoms such as agitation. Since it causes profound and long-term lymphocytopenia, irradiation of blood products to prevent graft-versus-host disease and antifungal prophylaxis is strongly recommended, from the start of treatment until 6 months after SCT or if SCT is not being performed until 6 months after the last administration of fludarabine.

G-CSF
This is a drug produced in E. coli by recombinant DNA technology that stimulates the production of neutrophils in the bone marrow. Side-effects occur occasionally, and concern beside local irritation mainly bone pain, vasculitis and thrombocytopenia. G-GSF
can be administered both subcutaneously and i.v., but for the latter administration special attention has to be given to the instructions for dilution and administration.

**Liposomal daunorubicin (DaunoXome®)**

This drug is the combination of an anthracycline with a unilamellar liposomal transport system. Daunorubicin is released slowly, and then mainly excreted in the bile, less so in the urine. Daunorubicin itself is metabolised to daunorubicinol, which is again mainly excreted in the urine and in the bile. The drug should be soluted in dextrose 5% only, because of agglutination in other solutions! Although not reported for DaunoXome®, the concomitant use of daunorubicin with heparin or dexamethasone i.v. is complicated by precipitation. Therefore, these drugs should not be used simultaneously i.v. with DaunoXome®. Because of its composition, care should be taken when using DaunoXome® concomitantly with parenteral nutritional lipid solutions or other liposomal products. Side-effects that have been described were nausea, vomiting, mucositis, hepatotoxicity and the dose-limiting toxicity is myelosuppression. Cardiotoxicity is rare, but has been documented both without (decreased left ventricular ejection fraction) and with clinical symptoms (congestive heart failure).

In case of a fractional shortening at echocardiography below 29%, DaunoXome® should not be given, at least not as part of this randomised trial. Another relative contraindication is a previous serious hypersensitivity reaction to DaunoXome® or any of its constituents.

In case of hepatic disturbances before the first administration of DaunoXome®, a reduction for all three doses is recommended:

- Transaminases >3-5 times upper normal limit (UNL): reduction of 25%.
- Transaminases >5-10 times UNL: reduction of 50%
- Transaminases >10 times UNL: not eligible

All three doses of DaunoXome® should be similar, in other words, no further reductions after the first dose.

**Melphalan**

This derivative of nitrogen mustard is a bifunctional alkylating agent. Consider dose-modifications in case of renal dysfunction. Main side-effects are nausea, vomiting, anorexia, myelosuppression, mucositis, diarrhea, rarely a hypersensitivity reaction, and hepatotoxicity after high-doses. The drug has been associated with pulmonary fibrosis, decreased fertility, and increased risk of secondary malignancies. Because melphalan competes with phenylalanine for cellular uptake, iv nutrition including amino acids should not be given one day before until at least two days following melphalan.

**Thioguanine**

This purine antagonist is an antimetabolite that is metabolised rapidly and then excreted renally. It may cause myelosuppression, nausea, vomiting, anorexia, mucositis, diarrhea, hepatotoxicity, and veno-occlusive disease.

**VP-16 (Etoposide)**

This inhibitor of DNA topoisomerase II should not be infused intravenously in less than 1 hour because of the risk of hypotension and anaphylaxis. The drug has several side-effects, including nausea, vomiting, alopecia, and myelosuppression. More rare side-effects are fever, headache, cholestasis, mucositis and peripheral neuropathy. Dose reduction is indicated in case of hepatic dysfunction.
Supportive Care

The treatment to be given is aggressive and immunosuppressive. Therefore, meticulous care is required in the management of patients entering the study (Riley 1999).

**General measures**

* **Venous access**: placement of a double lumen central venous catheter for administration of chemotherapy, nutrients, antibiotics and blood products is strongly advised.
* **Tumor lysis prevention**: to prevent tumor lysis syndrome, the patient should be well hydrated, alkalinized and placed on allopurinol (or uricozyme) before initiation of therapy (see also page 14)
* **Nutrition**: the combination of chemotherapy induced vomiting, mucositis, infection, and hemorrhage may result in significant weight loss. Progressive weight loss should be treated aggressively with supplemental enteral or parenteral nutrition; enteral feedings are preferred to parenteral; adjustment of the diet (p.e. no lactose or semi elementary) may be beneficial. Because melphalan competes with phenylalanine for cellular uptake, amino acids as part of parenteral nutrition should not be given one day before and two days following melphalan
* **Nill-by-mouth**: when enteral feedings must be withheld, protection of the gastric mucosa should be initiated
* **Antiemetics**: prophylactic antiemetic therapy should be instituted to prevent chemotherapy induced nausea and vomiting. Steroids should not be used in case of (suspected) fungal infections.
* **Mucositis**: meticulous oral hygiene is required; tooth brushing, hydrogen peroxide, saline and bicarbonate rinses; chlorhexidine solution; liberal use of pain medication for this condition is encouraged. Stomatitis due to herpes virus may be confused with drug induced mucositis; therefore, viral cultures should be obtained frequently. Anti-herpetic and anti-fungal therapy should be given as indicated.
* **Conjunctivitis prophylaxis**: during cytarabine and for 24-48 hours after completion, dexamethasone ophthalmic solution or isotears (q 6 hours) should be used to prevent cytarabine induced conjunctivitis.
* **Suppression of menstruation**: menstruating females should receive depo-provera or another suppressant during the entire course of the protocol until the platelet count is >40-50x10^9/l without transfusion support.
* **Vaccinations**: these should be postponed till after completion of treatment
* **Physical therapy**: timely exercises for preventing and maintaining optimal motor skills are recommended
* **Psychosocioeconomic support**: recommended
* **Prevention and treatment of pain**: according to local guidelines

**Hyperleukocytosis and metabolic derangement**

Patients with high peripheral blast counts (>50-100x10^9/l) and significant organomegaly have increased problems related to metabolic abnormalities, bleeding, and hyperviscosity. Therefore, special measures for reinduction are indicated and are described on page 14.
Prevention and treatment of infections

Prevention:
* mandatory hospitalization during and after treatment courses until the absolute neutrophil count is rising (and depending on the clinical condition)
* barrier nursing
* HEPA air filtration in the nursing room if available
* oral hygiene (see above)
* treatment of infectious foci before initiating therapy: ENT, dental prior to initiating therapy; multiple dental extractions may be necessary.
* surveillance cultures may be considered, according to local guidelines; routine culture of throat to detect the presence of penicillin-resistant streptococcus
* test for toxoplasmosis, hepatitis viruses, CMV, varicella and HSV antibodies
* bowel decontamination may be considered, according to local guidelines (Guiot 1983)
* prophylactic antibiotics in case of bacteriemia causing operations
* pneumocystis carinii prophylaxis with trimethoprim-sulfamethoxazole, e.g. 150/750 mg/m²/day for 3 consecutive days every week (Hughes 1987); alternatives: dapsone or aerosolized pentamidine
* because of the association of streptococcus viridans infection and high dose cytarabine, penicillin prophylaxis is recommended from completion of cytarabine administration until neutrophil recovery
* prophylactic treatment of fungal (including aspergillus) infections is recommended: e.g. with oral itraconazole (liquid!, 5-10 mg/kg/day in 1-2 doses, maximum 600 mg/day)
* at the time of stem cell transplantation, prophylactic acyclovir for patients with positive HSV titers
* administration of zoster hyper immune globulin within 48 hours after a real varicella contact
* Restarting G-CSF at day 15 of each of both reinduction courses is recommended to reduce the duration of neutropenia. G-CSF should be stopped again upon neutrophil recovery, as defined by local guidelines.

Treatment in case of fever and neutropenia:
Because of the high risk of serious infectious complications, patients developing fever should be treated for presumed sepsis with broad spectrum antibiotics. Because of the association of high dose cytarabine with streptococcus viridans infection and because of the possibility of a contaminated central venous line (staphylococci) the antibiotic regimen should include drugs specific for the treatment of an infection with gram-positive bacteria (vancomycin, teicoplanin). Antifungal therapy, such as amphotericin B should be administered when defervescence does not occur within 3-5 days.

AML treatment is associated with a high risk of pulmonary aspergillus; however, pulmonary infiltrates can also be caused by bacteria, virus (CMV), legionella and PCP; therefore, aggressive diagnostic measures including (repeated) high resolution CT scans, bronchoscopy with biopsy or bronchoalveolar lavage, and open lung biopsy, should timely be contemplated.

G-CSF, if not given already, may be considered to reduce the duration of neutropenia, in patients with life-threatening bacterial or fungal infections.

Treatment in case of a documented infection:
The empiric treatment may be adjusted when a specific cause for the infectious symptoms is found, although broad-spectrum antibiotics remain (at least initially) indicated in case of neutropenia.
G-CSF
Granulocyte colony stimulating factor (G-CSF) will be used as part of the FLAG regimen from the day before starting fludarabine until the first day after fludarabine (for a total of 6 days). Adding G-CSF to FLA has been decided because the pilot-studies were done with FLAG and because of several papers describing favorable interactions of G-CSF with fludarabine and cytarabine (Braess 2000, Gandhi 1995; see also “background and introduction”). After the discontinuation it is recommended to restart G-CSF at day 15 of each reinduction course in order to shorten the period of neutropenia in these patients at high risk for severe infections, of which the occurrence is correlated with the duration of neutropenia. G-CSF appears safe in that it had no negative impact on the prognosis of adult AML in 2 large randomised studies (Godwin 1998, Heil 1997), and its use reduced duration of neutropenia, fever, hospitalisation, and use of antibiotics and antifungals (Chen 1998, Godwin 1998, Heil 1997). G-CSF should be stopped again upon neutrophil recovery, as locally defined. The use of G-CSF before day 15 of each course for prevention or treatment of chemotherapy induced fever and neutropenia is considered useless, although this has not been studied in this particular setting.

Transfusion support
* Bleeding due to thrombocytopenia should be treated promptly; during the induction period and throughout admissions for fever and neutropenia, it is recommended that the platelet count be maintained >15-30x10^9/l. Higher tresholds may be indicated, for instance in case of lumbar punctures and surgery.
* Packed red blood cell transfusions should be used to correct hypovolemia from blood loss or pre-existing anemia (Hb < 5 mmol/l = 8,0 g/dl). Higher threshold levels may be indicated in case of pneumonitis or (imminent) bleeding or other causes of a compromised clinical condition.
* The blood products should be leukocyte-depleted to prevent HLA sensibilization; leukocyte-depleted blood products can be considered CMV-safe.
* To prevent transfusion related graft versus host disease, cellular blood products should be irradiated (25 Gy) during and at least till 6 months after fludarabine, in case of (imminent) lymphopenia (< 500/ml), and from 2 weeks before till at least 6 months after stem cell transplantation.
* In case of allo-immunization HLA-matched platelets may be required.

Mesna
Cyclophosphamid induced hemorrhagic cystitis should be prevented by the use of mesna and forced diuresis.
Clinical evaluation, laboratory tests and follow-up

1. **Before the start of the treatment** the following parameters need to be obtained:
   - medical history and physical examination
   - performance status (depending on age, either the Karnofsky or Lansky Performance scale)
   - complete blood count (hemoglobin, platelets, white blood cell count and differentiation). This should be determined within 2 days prior to the first reinduction course.
   - serum chemistry: creatinine, sodium, potassium, calcium, phosphate, ALAT, bilirubin, uric acid, LDH, CK, glucose, total protein, albumin; clotting (APTT, PT, fibrinogen).
   - HLA typing (if not done previously)
   - recent information on the viral serology (antibodies: EBV, HSV, CMV, PVB19, HAV, HBV, HCV, HIV; PCR: CMV, HCV) and IgG and IgA levels should be available.
   - bone marrow examination (marrow cellularity and percentage of blasts). Perform a biopsy in case of a dry tap. This should be performed within 3 days prior to the first reinduction course. Bone marrow should also be sent to the reference laboratory for confirmation of the diagnosis, and should be studied for immunophenotype and cytogenetics. Please consider to participate in research studies (MRD, drug resistance, etc.: see information on page 36 and Appendices).
   - lumbar puncture (quantification of cells and protein, pathological examination), also to be done within 3 days from the start of reinduction chemotherapy.
   - urine (protein, glucose, ery’s)
   - Chest X-Ray
   - Abdominal ultrasound
   - MRI (=NMR) of the brain in case of (the suspicion on) localised symptomatic CNS disease
   - Electrocardiogram
   - Echocardiography (please refer to the more comprehensive guidelines below)
   - Lung function test (optional)

2. **During initial treatment, i.e. the 1st course**, to monitor the disease:
   - At least twice weekly a complete hematological count and serum chemistry are needed as described above
   - a twice or more weekly physical examination is performed
   - at day +15 after the start of reinduction treatment a bone marrow sample is obtained to determine early clinical response, which may be different between both treatment arms and which may have prognostic significance. Slides should also be send to the reference laboratory for morphological (and flowcytometrical) examination, together with peripheral blood slides.
3. **Before the start of each treatment course** the remission status and toxicity should be determined.
- bone marrow and peripheral blood morphology (with central review by the reference laboratories)
- lumbar punction (quantification of cells and protein, pathological examination of the CSF)
- physical examination
- complete blood count and blood chemistry (see above)
- echocardiography (see page 30)
- the patient should be followed for events (relapse/death), both after discontinuation of treatment according to this protocol and after proceeding to bone marrow transplantation.

Toxicity data must be determined according to the NCI Common Toxicity Criteria (Appendix J) after each treatment course, with special attention for cardiac abnormalities. The Therapy Checklist must be documented carefully (Appendix K).

Serious adverse events (defined on page 31) must be reported immediately (Appendix C) to the central data office in Münster (as well as to the own data center).

### Table 1. Investigations before, during and after initial treatment.

<table>
<thead>
<tr>
<th></th>
<th>Before start of treatment</th>
<th>Weekly</th>
<th>Day 15 after start of course I</th>
<th>Weekly</th>
<th>Day 28-42 after start of course I</th>
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<tbody>
<tr>
<td>Medical history</td>
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<tr>
<td>Physical examination</td>
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<td>Twice or more</td>
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<td>Performance status</td>
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<td>Therapy checklist</td>
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<tr>
<td>Hematology (see text)</td>
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<td>Chemistry (see text)</td>
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<td>Bone marrow punction¹</td>
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<td>Send BM and PB to</td>
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<td>reference laboratory</td>
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<td>Send bone marrow to</td>
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<td>Amsterdam for drug</td>
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<td>resistance studies</td>
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<td>BM and PB for MRD</td>
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<td>(central laboratories; research)</td>
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<td>Lumbar puncture</td>
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<td>Echocardiography</td>
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¹In case of a dry tap, perform a biopsy
Cardiotoxicity monitoring

**Why**: to determine possible short- and long-term cardiotoxicity, and to detect any differences in the incidence and severity of cardiotoxicity in patients who did or did not receive liposomal daunorubicin.

**When**: In both treatment arms and in all randomised patients, echocardiography must be performed within 1 week before start of reinduction treatment, within 1 week prior to the second course (FLAG), within 1 week prior to the conditioning regimen as part of SCT, and 6 months after SCT. In case of a normal FS of >28%, then repeat echocardiography every 3 years (earlier if clinically indicated). In case of an abnormal FS, then repeat echocardiography at least every year. Additional echocardiography (and other monitoring) may be indicated in case of growth spurt, involvement in regular strenuous exercise, growth hormone and/or sex hormone replacement therapy and in case of pregnancy.

**How**: M-mode echo measurements according to the American Heart Association guidelines. Whenever possible, the patient should be afebrile and have a normal hemoglobin (>9 g/dl, >5.6 mmol/l). If an ECG is not recorded simultaneously with M-mode echo, greatest and least LV dimensions should be used for diastolic and systolic measurements respectively. Please note the date of each scan, along with the height, body weight, and three blood pressure measurements (preferably obtained during the last part or immediately after the echo; ensure that the cuff is in place before starting the echocardiography), as well as initials, gender, race, and identification number.

Please save all the information of one patient on one (or more) tape (VHS/S-VHS) to allow central review at a later stage. At least, save the recordings on tape, and note which type of echo-machine was used.

**What**:
- Attach ECG
- Baseline echocardiogram: apical 4 chamber view
  apical 5 chamber view (with aorta)
  short axis left ventricle (papillary muscle level)
  short axis left ventricle (aortic valve/pulmonary artery level)
- Baseline colour Doppler: apical 4 chamber (mitral, tricuspid, aortic flows)
- Pulsed Doppler: aortic flow (measurement of LVET)
  mitral valve (tips of mitral valve leaflets)
- M-mode: left ventricle – parasternal long axis

M-mode cursor perpendicular to interventricular septum in a plane where the mitral valve and aorta (aortic valve) are visible
M-mode cursor immediately below tips of mitral valve
Confirm on tape that the M-mode cursor is positioned correctly
M-mode recording of aortic valve opening (for LVET) if aortic Doppler was not obtained (see above)
Record a minimum of 10 good quality cardiac cycles
Record blood pressure (see above)

**Fractional Shortening** will be used as main measurement of cardiac function.
Criteria of response and other definitions

CNS disease
$\geq 5$ white blood cells/mm$^3$ (ie, $\geq 5/\mu l$, or $\geq 15/3 \mu l$) and unequivocal evidence of blasts on cytopsin examination, and/or clinical (seizures, cranial nerve palsy and symptoms of increased cranial pressure or other signs/symptoms not readily explained by another disease) and/or radiological evidence of leukemic infiltration in the central nervous system. In all of the symptomatic cases, a MRI should be made. CSF not evaluable in case of $>50/3$ erythrocytes.

Complete remission
$\leq 5\%$ leukemic blasts in the bone marrow with signs in the BM of normal hematopoiesis and with clear signs of regeneration of normal blood cell production in the peripheral blood (platelets $> 50\times 10^9/l$ without transfusions, neutrophils $>1.0\times 10^9/l$), and no leukemic cells in the PB or anywhere else.

Death, early
Death during the first 2 months of treatment (ie, before the time that complete remission could have been documented). The cause of death (disease, therapy or both) should be recorded as well as the last known percentage of blasts in the BM. Only patients that were randomised will be included for this analysis

Death, toxic
Death due to treatment-related complications, and not caused by the leukemia itself

Partial remission
No complete remission, BM blasts $> 5 \leq 20\%$ and/or no regeneration

Refractory disease = non-responder
More than $20\%$ of blasts in the bone marrow after 1 or 2 complete blocks of chemotherapy, and/or elsewhere documented leukemic cells after 2 complete blocks of chemotherapy

Relapse
After a documented complete remission, the recurrence of $\geq10\%$ unequivocal leukemic cells (occasionally, 10-20% blasts in the BM does not represent a relapse!) in a representative bone marrow, and/or evidence of leukemic infiltration or recurrence at any site. CSF with $<5$ cells/mm$^3$ but with blasts on the cytopsin examination should be investigated repeatedly, as should the BM in case of $>5<10\%$ leukemic cells and in case of doubt otherwise.

Relapse, early first
Relapse within 1 year from initial diagnosis

Relapse, late first
Relapse occurring 1 year or later from initial diagnosis

Serious adverse event
Death, life-threatening or permanently disabling event, and/or need for hospitalization or prolongation of hospitalization
Statistical design and analysis

This concerns an international multicenter open label randomised phase III clinical study on the treatment of children with relapsed or refractory AML. As can be seen from the overview on page 2, a large number of groups from all over the world will collaborate.

The main end point is the percentage of BM blasts (yes or no >20%) after one block of Chemotherapy (FLAG or FLAG/DaunoXome®), which will be compared between both arms.

Secondary end points are:

- Toxicity will be measured by duration of bone marrow aplasia and by CTC-NCI grading for mucosal toxicity, short- and long-term cardiotoxicity (see page 30) and other NCI-CTC scales which are considered to be relevant in relapsed AML (see Appendix J). Again, toxicity will be compared between both treatment arms.
- Efficacy will also be determined by: percentage of blasts in the day 15 bone marrow, time to peripheral blood blast clearance, CR rate after 2 courses of (re-) induction, percentage of patients with performance of SCT, overall survival (event: death from any cause), event-free survival (events: death from any cause, non-response, relapse or second malignancy) and disease-free survival (DFS, events: death from any cause, relapse or second malignancy). Survival times will be calculated from date of entry on the protocol (randomisation) (or date of CR for DFS) to last follow up or next event. These parameters of clinical treatment response will be compared between both arms.

The analysis of the main end-point will be performed on all randomized patients according to the intent-to-treat principle. The difference in response rate (yes or no >20% blasts in the BM) will be tested with a logistic regression model taking into consideration time from first diagnosis of AML to relapse and whether patients had primary refractory AML or relapsed disease.

The expected number of patients that will be included in this trial is 100/year, based on the participation of the above mentioned groups. The study duration will be 4 years, or less in case of a higher accrual rate. Assuming a randomisation rate of 90% about 360 patients will be randomised. The power will be 82% to detect an increase in the CR rate from 50% to 65% (two-sided test, alpha=5%).

Final analysis will be performed 24 months after the inclusion of the last patient. Two interim analyses for efficacy are planned after 150 and 300 patients. The O’Brien and Fleming rules will be followed for alpha-correction. If the increase in CR rate is demonstrated already at one of these interim analyses, the international study coordinators and statistician will consult the data monitoring committee.

The absolute death rate observed in each arm (and globally in the whole population) will be compared to a reference rate in order to detect an absolute excess of toxic deaths. All patients who die within the first two months of therapy or have a follow up of at least two months will be included to calculate the rate of early death (see table, left column). If with a given number of patients the number of deaths should reach the limit (see table, right column), the Data Monitoring Committee and the study writing committee have to decide about stopping or continuing the study. Based on previous experience, we choose the
following parameters: $p_0 = 10\%$ and $p_1 = 20\%$ with $\alpha = 5\%$ and $\beta = 1\%$. This means that the risk to wrongly conclude that there is an excess of toxic deaths (whereas the real rate is equal to $p_0 \leq 10$) is equal to $\alpha = 5\%$. On the other hand, the power to detect an excess of toxic deaths (if the real rate is equal to $p_1 = 20$) is equal to $1-\beta = 99\%$. Major adverse events will be monitored continuously.

<table>
<thead>
<tr>
<th>N (death within/follow-up of at least two months)</th>
<th>N (death within 2 months of therapy)</th>
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<td>-9</td>
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<td>24</td>
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Data management, registration and randomisation

Each participating group will refer to their clinical contact person and to the usual network of clinical centers, data center and experts (statistician, laboratories, etc) for the application of this protocol. Appendix H mentions the names and e-mail addresses of the contact persons (clinical and data management) of each participating group. The international study coordinator, vice coordinator and study statistician will act as a coordination unit for the monitoring and exchange of information and for the pooling of the data.

The set of data to be collected and pooled by the participants are listed in Appendix I. Each group may:

• design its own data collection forms for this protocol according to its own standards, but in such a way that the forms include the data items listed in Appendix I; Please note, however, that toxicity collection after each course should be done using the common form (listed in Appendix J)
• centralize the forms for quality checks in its own data center, according to the approach routinely used in the group.
• decide wether to input data in their own data base and send data as a file for pooling (at least every 6 months) or to send each checked and corrected forms to the operating center in Munster

In summary, the major requirements that each group will have to ask to each of its clinical oncology centers are:
1. To register at the data center each patient with relapsed or refractory AML, regardless of whether the patient will subsequently enter the protocol (Appendix B). This is necessary in order to know which percentage of eligible patients is treated according to the protocol. Registration should be done as soon as possible after diagnosis; entry on this study can only be done with informed consent and after completing the randomisation procedure.
2. To send at least every 6 months the forms on diagnosis, response, randomization, treatment, toxicity and events as soon as they can be completed, to the central data office (see addresses).

Please note that serious adverse events must be reported immediately to the central data office in Münster (as well as to the own data center).

Data pooling
A common coding system and format has been made, for use by the data managers and/or statisticians, in order to minimize mistakes in data exchange. This coding system is defined for the purpose of data pooling only: it is required that a data set including each registered patient is prepared with this format by the data managers and/or statisticians of each group. The data have to be sent every 6 months for pooling to the operating center, in a file structured according to a file that will be forwarded separately to the data managers/statisticians, or on forms. The operating center, in collaboration with the clinical contact person and the data manager/statistician of each group, pools the data and circulates a report on these data.
Data Monitoring Committee (DMC)
Such a committee (two clinicians and one statistician) will be installed. Members of the DMC are experienced researchers not involved in the trial who will be responsible for providing the principal investigators with guidance on the trial conduction and, in case of problems, on whether the trial should be stopped, modified or continued.

Registration and Randomisation Procedure
There will be a continuous possibility (7 days/week, 24 hours/day) of computerised registration and randomisation. The local/national representatives for data management will be instructed by the central data office (Martin Zimmermann) about the procedure. After that, these local data managers will distribute login names and passwords to all participating centers of their group. Then, each individual center will have the possibility to randomise a new patient using the internet. Alternatively, the randomisation can be done centrally by the local group/national data manager, or the central data office in Münster. Each group is allowed to organise the randomisation of their own patients, but it must be balanced for early (within one year from initial diagnosis) and later relapse. The two addresses (the second one being the relevant one for the individual centers) are:

http://www.mh-hannover.de/institute/biometrie/AML/LoginCoordinator.html
http://www.mh-hannover.de/institute/biometrie/AML/LoginInvestigator.html

There will be a back-up procedure if the internet system is not available. In these circumstances, please consult your local representative for data management (see appendix H), or if not available the central data office for this study in Münster (page 2).
Publication and other policies

Authors on abstracts and manuscripts:
- Final main publications to be written by international study coordinators
- Members of the writing committee of the protocol will be included
- Clinical contact persons of each of the participating groups, if a total of at least 10 patients has been included from that group in the Relapsed AML 2001/01 study, will be included
- International statistician
- Manuscripts concerning add-on studies only will include those who made a significant contribution to that particular study, according to international guidelines for authorship

Acknowledgements in manuscripts:
- All of those who made a significant contribution to the preparation, execution and/or analysis of the study, not included as a co-author, will be mentioned in the acknowledgements. Examples are: data managers of each of the participating groups, and clinical representatives of groups who did not fulfill the requirements for co-authorship.

Other guidelines:
- Any publication, abstract or presentation based on patients included in the international studies: approval is required by both international study coordinators
- Publications (including abstracts, presentations etc.) comparing the randomised treatment arms and/or concerning study end-points by individual groups are not allowed, until final results of the international studies have been published
- Participating groups are obliged to send all required patient data every 6 months to the central data office in Münster. Groups who fail to do so will loose their status as collaborating group.

Ethical considerations

The appendices contain an example of patient/parent information and an informed consent form. This will be obligatory to have, however, local policies will determine final details about these procedures. If requested, patient insurance must be organised by the local/national groups and/or by the individual study centers.
Translational research, add-on studies

The participation in these studies is voluntarily for study groups, individual centers and patients and their parents. However, participation is encouraged. More detailed (also practical) information is given in the appendices.

At the time of this draft, these are the applied study proposals:
1. Minimal residual disease (central laboratories, coordinated by D. Reinhardt, Munster; please refer to appendix F)
   - flowcytometry
   - molecular biology
2. Cellular drug resistance and biology of the leukemic cells (G.J.L. Kaspers, Amsterdam; please refer to appendix E)
   - MTT and clonogenic assays
   - Cell biological features
   - Antigenicity and autologous antileukemic activity
3. DaunoXome® pharmacokinetics (J. Boos; Munster; please refer to appendix G)
4. Flt-3 (Ch.M. Zwaan, Amsterdam; included in appendix E)
   - The Flt-3 gene encodes a tyrosine kinase receptor that regulates proliferation and differentiation of hematopoietic stem cells. De novo AML patients with an internal tandem duplication (ITD) of Flt-3 on their AML cells are suggested to have a bad prognosis compared with children without such an ITD (Meshinchi et al., Blood 1999, 94: p504a, abstract #2258; Zwaan et al., Leukemia 2001, 15: 493, abstract O51), but also without ITD’s the Flt-3 gene can be autophosphorylated and mutated. Tyrosine kinase inhibitors will also be tested in vitro in relation to Flt-3 gene expression. These studies will be done in the setting of add-on study 2 (see above).

Support

This study is financially supported by Gilead Sciences (producer of liposomal daunorubicin, DaunoXome®) and Schering AG (producer of fludarabine, Fludara®), which enabled the appointment of a (pre-)clinical investigator and an international data manager.
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## Appendices

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Appendix A  Patient/parent information and informed consent

This is an example and has to be re-written according to local institution format and policies.

This form informs you on a clinical trial. Clinical trials include only those patients who choose to take part. Please take your time to decide if you want to participate in this study or not.

Patient/parent information with respect to: “A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2001/01”

You/your child has unfortunately been diagnosed to have refractory or relapsed acute myeloid leukemia (AML). The chance of cure now is lower than it was at the moment of the initial diagnosis. The optimal treatment of refractory and relapsed AML in children is unknown. However, there are clear indications that the combination of fludarabine, cytarabine (at high doses), and granulocyte-colony stimulating factor (FLAG) may be effective. In addition, so-called anthracyclines may still be of value in relapsed/refractory AML. Its use, however, is limited by the cardiotoxicity that these drugs may have when given at relatively high cumulative doses. Your child has been treated with anthracyclines and/or related drugs already. Therefore, we are reluctant to give an anthracycline again. However, there are strong indications that a novel formula of daunorubicin, so-called liposomal daunorubicin (DaunoXome®), has less cardiotoxicity than conventional daunorubicin, but has similar or even increased antileukemic activity. It is unknown whether liposomal daunorubicin added to FLAG has an additional value in terms of an increased chance of cure. Therefore, we want to compare both regimes, ie, FLAG and FLAG plus liposomal daunorubicin, in the first course of the treatment of your/your child’s current disease. In order to be able to make a fair and unbiased comparison, fate should decide whether you/your child will be treated with FLAG or FLAG plus liposomal daunorubicin. We call this a randomised study. Because about 400 patients should be included in this study, and because refractory/relapsed AML is rare in children, the study will be done in collaboration with most European countries and with an American center. After the first course of treatment, all children should receive a second course of FLAG. However, if a patient does not respond to the first course of so-called reinduction treatment, further treatment according to this protocol is considered useless, and you should talk with your doctor about further treatment options. After the 2 reinduction courses, patients should directly proceed to a stem cell transplantation if that is actually possible. Otherwise, a 3rd course of treatment will be given, followed by the stem cell transplantation. However, for patients who are not in so-called complete remission after the 2 reinduction courses, further treatment according to this protocol is again considered useless and further treatment options should be discussed with your doctor. The type of donor that will be used depends on the availability of a donor and the type of AML (relapsed or refractory, early or late relapse) that you/your child has. You will receive additional information on the stem cell transplantation later on. The possible side-effects of this chemotherapeutic treatment will not be different from the side-effects associated with the treatment of the initial AML. The efficacy and toxicity of the treatment will be carefully monitored. Potential cardiotoxicity will be monitored using regular echocardiography in both patient groups.
In order to make progress in the treatment of AML, additional scientific research is necessary. Therefore, we ask for your permission to use left-over bone marrow and/or blood that was taken for necessary clinical tests. Sometimes cells are left-over, and sometimes not. The use of material that is left-over does not require additional procedures. We would like to study e.g. the presence of so-called minimal residual disease (AML cells that are not visible using the normal light microscope) in the course of the treatment, the detailed characteristics of your/your child’s AML cells, and the cytotoxicity of old and new drugs on your/your child’s AML cells as measured in the laboratory. Scientific studies that would require additional procedures will be discussed with you separately, and information will also be given separately. All these scientific studies do not affect the treatment of you/your child. You should feel free to decide not to participate in these additional scientific studies, at any time. Such a decision will in no way affect the treatment of you/your child. Finally, all information that will be obtained in this study (including the additional scientific studies) will be analysed and reported anonymously, in order to protect your/your child’s privacy.
Informed consent form with respect to: “A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2001/01”

☐ Yes, I have been properly informed on treatment with this protocol, and I have been explained that the study aims at investigating the efficacy and toxicity of liposomal daunorubicin (DaunoXome®) in relapsed/refractory childhood AML, and aims to offer a comprehensive treatment of this disease. I have received a copy of the patient information sheet.

☐ Yes, I agree with treatment according to the protocol, entitled: “A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2001/01”

☐ Yes, I agree with randomisation for the 1st course of reinduction chemotherapy, either “FLAG” or “FLAG plus DaunoXome®”

☐ Yes, I agree to have the above mentioned additional scientific studies done on extra cells taken from my bone marrow and peripheral blood, as explained in the patient/parent information. It concerns cells that were taken at necessary routine tests, but that were left-over (which sometimes does and sometimes does not occur)

☐ Yes, I agree to have the required information, both on my disease as well as on the results of the treatment, transferred to the investigators, to allow an evaluation of the results of the treatment, and for scientific research. However, my (my child's) privacy is guaranteed and data will be transferred and reported anonymously.

☐ Yes, I know that I may withdraw my cooperation at any time without having to provide an explanation and without experiencing any disadvantage from it.

Place and date

Hospital

Signature parents

Signature patient (if applicable)

Signature pediatric oncologist
Appendix B  Registration form and Randomisation Procedure

ONLY TO BE USED IF THE INTERNET PROCEDURE IS NOT AVAILABLE

Whenever possible, please use the internet for registration and randomisation:

http://www.mh-hannover.de/institute/biometrie/AML/LoginCoordinator.html
http://www.mh-hannover.de/institute/biometrie/AML/LoginInvestigator.html

To: Local Data Office (see appendix H) for the Relapsed AML 2001/01 Study

FAX nr: E-mail:

Θ This is to inform our local data office for this study in relapsed/refractory AML, that we will include a patient (please fill in the details below) in this randomised phase III study, entitled:

“A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2001/01”

I declare that the patient I will include is eligible for the Relapsed AML 2001/01 protocol for relapsed or refractory AML. I have obtained formal written informed consent, which I will forward soon.

Θ This is to inform the principal investigators of this study in relapsed/refractory AML, that we will not include a patient (please fill in the details below) with relapsed/refractory AML in this randomised phase III study, because: a) randomisation was refused by the patient/parents, b) randomisation was refused by the doctor, c) the patient was not eligible, d) for other reasons (please indicate the reason) (please encircle)

Treating Pediatric Oncologist:

Name:
Center:
Phone:
Fax:
E-mail:

Patient data: a) primary refractory disease
(please encircle) b) refractory to other reinduction protocol after relapse
b) first relapse 1) early 2) late
c) subsequent relapse

Initials:
Date of birth:

PLEASE CONTACT YOUR LOCAL OR THE INTERNATIONAL DATA OFFICE FOR RANDOMISATION IN CASE THE INTERNET PROCEDURE IS NOT AVAILABLE

31 July 01/45
Appendix C

Serious adverse event form

To: International Data Office study Relapsed AML 2001/01
FAX: +49 – 251 835 6489
E-mail: zimmermann.martin@mh-hannover.de

And to: Local Data Office of the Own Group
FAX:
E-mail:

Date:

This is to inform you that we have observed a serious adverse event in a patient with relapsed or refractory AML, who was treated according to the international multicentre phase III study, entitled:

“A randomised phase III study on the treatment of children and adolescents with refractory or relapsed acute myeloid leukemia – TRIAL Relapsed AML 2001/01”

Reporting pediatric oncologist/treating physician:

Name:

Center:

Phone:

Fax:

E-mail:

Please indicate:

- death Y/N
- life-threatening Y/N
- permanently disabling Y/N
- need for hospitalization or prolongation of hospitalization Y/N

Comments:

31 July 01/46
## Appendix D  Performance scales

### Lansky score: 0-16 years

<table>
<thead>
<tr>
<th>Activity</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully active</td>
<td>100</td>
</tr>
<tr>
<td>Minor restriction in normal physical activity</td>
<td>90</td>
</tr>
<tr>
<td>Active, but tires more quickly</td>
<td>80</td>
</tr>
<tr>
<td>Both greater restriction and less time spent in active play</td>
<td>70</td>
</tr>
<tr>
<td>Minimal active play, busy with quieter activities</td>
<td>60</td>
</tr>
<tr>
<td>Gets dressed, but no active play, able to participate in all quiet play and activities</td>
<td>50</td>
</tr>
<tr>
<td>Mostly in bed, participates in quiet activities</td>
<td>40</td>
</tr>
<tr>
<td>In bed, needs assistance even for quiet play</td>
<td>30</td>
</tr>
<tr>
<td>Often sleeping, play limited to passive activity</td>
<td>20</td>
</tr>
<tr>
<td>No play, does not get out of bed</td>
<td>10</td>
</tr>
<tr>
<td>Unresponsive</td>
<td>0</td>
</tr>
</tbody>
</table>

### Karnofsky score: 16 years and older

<table>
<thead>
<tr>
<th>Activity</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, no complaints, no evidence of disease</td>
<td>100</td>
</tr>
<tr>
<td>Able to carry on normal activities</td>
<td>90</td>
</tr>
<tr>
<td>Normal activity with effort</td>
<td>80</td>
</tr>
<tr>
<td>Cares for self, unable to carry on normal activity or to do active work</td>
<td>70</td>
</tr>
<tr>
<td>Requires occasional assistance, is able to care for most of own needs</td>
<td>60</td>
</tr>
<tr>
<td>Requires considerable assistance, frequent medical care</td>
<td>50</td>
</tr>
<tr>
<td>Disabled, requires special care/assistance</td>
<td>40</td>
</tr>
<tr>
<td>Severely disabled, hospitalization</td>
<td>30</td>
</tr>
<tr>
<td>Hospitalization, very sick, active treatment</td>
<td>20</td>
</tr>
<tr>
<td>Moribund, fatal processes in progression</td>
<td>10</td>
</tr>
<tr>
<td>Dead</td>
<td>0</td>
</tr>
</tbody>
</table>
Appendix E  Cellular drug resistance testing

Background: The success or failure of chemotherapy essentially depends on three factors: 1) pharmacokinetics, 2) cellular drug resistance (or, at the other end of the spectrum, sensitivity), and 3) relapse potential of minimal residual leukemic cells. The relatively poor prognosis of acute myeloid leukemia (AML), and of relapsed and refractory AML in particular, indicates the need for detailed studies of these three factors in AML. Our group in Amsterdam has focussed on preclinical research on cellular drug resistance in childhood leukemia. We demonstrated the clinical relevance of cellular drug resistance testing in both acute lymphoblastic leukemia (Den Boer 1998; Kaspers 1995, 1997, 1998, Klumper 1995; Ramakers-van Woerden 2000; Rots 1999) and in AML (Kaspers 1999, Zwaan 2000, Rots 2001). The relapse potential may also be related to an escape from immune surveillance, associated with differences in antigenicity, and MHC- adhesion- and co-stimulatory molecule expression on AML cells. Finally, novel treatment modalities emerge, such as targeted therapy with monoclonal antibody-labeled cytotoxic agents, diphteria toxins (DT), tyrosine kinase inhibitors, and histon deacetylase and farnesyl transferase inhibitors.

Objectives: In the setting of our research on cellular drug resistance in relation to cell biological features in childhood leukemia, we want to explore mechanisms of resistance to conventional agents such as cytarabine, and ways to modulate or circumvent such resistance. In addition, we want to study the correlation between exposure of AML cells to immune system activating cytokines and cellular drug resistance, in perspective of the antigenicity and expression of MHC-, adhesion and co-stimulatory molecules of these AML cells, and autologous anti-leukemic activity. Finally, we will study the differences between patient samples in sensitivity to novel agents, such as tyrosine kinase inhibitors, in relation to cell biological features of the AML cells. Examples are the in vitro antileukemic activity of STI571 and SU5416 and mutations and autophosphorylation of c-kit, the activity of a novel Flt3-DT and abnormalities in the Flt3 gene/protein, and the activity of R115777 and ras-protein activation. Interactions between these novel agents and conventional drugs will also be investigated.

Methods: Cellular drug resistance assays such as the MTT assay, DiSC assay and clonogenic assay will be used. FACS and immunocytochemistry will be used to determine expression of MHC-, adhesion and co-stimulatory molecules, and a sandwich ELISA for measurement of cytokines in supernatants of AML cells. Autologous cell-mediated antileukemic cytotoxicity will be determined in a limited number of samples by flow cytometry. In addition, Western blotting and PCR techniques will be applied, as well as a limited number of DNA micro-array analyses. For certain mechanistic studies, HPLC and radiochemical assays will be used, while SSCP and DNA sequencing will be used to determine mutations and polymorphisms.

Logistics: Material will be used that is obtained at clinically necessary sampling of blood and bone marrow, for the diagnosis and follow-up of a new or relapsed or refractory AML. Therefore, additional procedures are not required. Preferably fresh bone marrow (3-5 ml) and/or blood (8-16 ml) should be sent to our laboratory in Amsterdam by courier service. One phone call to our laboratory is enough, we will then make further arrangements! The preclinical research is free of charge, if necessary we can also pay the transport costs. Please also refer to the information and forms below.

Literature:
1. ANNOUNCEMENT
   - inform us preferably before, otherwise immediately after, sampling by calling our laboratory: +31 20 444 2977 or -2683 or –3247 (if not present: 06 – 201 24 669)

2. SAMPLE
   - use preferably the standard preheparinized tubes that are supplied by our laboratory in Amsterdam
   - 3-5 ml bone marrow and/or 8-16 ml peripheral blood
   - keep the tubes stored at room temperature
   - if no preheparinized tubes are available, use a maximum of 20 IU heparin per ml blood
   - new standard preheparinized tubes will be sent back directly

3. SHIPMENT
   - we will instruct TNT Worldwide Express (our courier service) to pick up the sample at your hospital
   - the TNT service will contact you about the time and location of the pick up
   - fill in the invoice form and include 2 copies
   - fill in the patient documentation form
   - do not use the box “priority delivery” on the freight bill of the courier

4. PAYMENT
   - the drug resistance testing is free of charge

Research Laboratory of Pediatric Oncology, 4 Noord
VU medical center
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands

phone: +31 20 444 2977 or -2683 or -3247
fax: +31 20 444 2684
E-mail: gjl.kaspers@vumc.nl
(please note: new e-mail address)
INVOICE  BLOOD SAMPLES  FRAGILE

Date: .................................  Invoice no: .................................

To:  VU medical center
    Research laboratorium kinderoncologie, 4Noord
    De Boelelaan 1117
    NL-1081 HV Amsterdam
    The Netherlands

Afleveren op werkdagen: de postkamer van het VU ziekenhuis
in het weekend: de portier Hoofdingang VU ziekenhuis

Bij aflevering direct het HOI-laboratorium bellen:  tst 42977 of 42683 of 43247
(bij geen gehoor: mobiel 06 - 201 24669)

Pakje bij kamertemperatuur bewaren

From:  Name of contact person:

Hospital:

Department:

Address:

ZIPcode and City:

Phone:

Telefax:

E-mail:

Description of contents:

This package contains human blood samples for research purpose only

Value:  DM 10.00 or GBP 5.00

Weight:  less than 1.5 kg

Country of origin (please specify):

Storage conditions  room temperature
(Please, enclose this form with the sample)

**Patient Documentation**

Name of the patient: __________________________________________
Registration no: __________________________________________
Date of birth: __________________________________________
gender: male / female

**Leukemia Documentation**

(please encircle or specify)

* did the patient receive any treatment in the two weeks before this sample was taken, e.g Allopurinol, antibiotics, Prednisolone, other cytostatic drugs:
  no / yes
if yes, please indicate which agents have been given:

* Please specify FAB-type:

* sample taken: non-responder / 1st relapse / 2nd or later relapse

* white blood cell count on the day of sampling:

REMARKS:

__________________________________________________________________________

__________________________________________________________________________
Appendix F

Minimal Residual Disease

“Minimal residual disease” (MRD) in relapsed/refractory AML in children

Background: Remission in AML is defined by morphological criteria. Persistent leukemic blasts less than 5% are not detectable. Therefore methods with a higher sensitivity are needed to enable a better definition of remission. By PCR a sensitivity of \(10^{-5}\) to \(10^{-4}\) is possible, unfortunately only in 15% to 30% of the patients a molecular marker could be identified. By contrast flow cytometry enables the definition of a leukemia-specific immunophenotype in about 80 to 90%. However, sensitivity is much lower \((10^{-2} - 10^{-3})\). A more precise detection might have implications on treatment strategies and identify patients with poor prognosis especially prior to stem cell transplantation (SCT). A standardized method is necessary to reveal comparable data. The panel proposed below is used in the AML-BFM 98 MRD study. This add-on study is for research only, because the prognostic relevance has not been defined so far.

Objectives:

a) Prognostic relevance of a fast blast clearance during re-induction treatment

b) Predictive value of MRD pre-SCT for outcome

Methods:

MRD should be analyzed at six time points during treatment course.

1. Definition of the leukemic immunophenotype at diagnosis of relapse (day 56-64)
2. MRD after first re-induction block (day 15)
3. MRD in regeneration (day 28)
4. MRD after second re-induction block (day 56-64)
5. MRD pre SCT (day –7/8)
6. MRD post SCT (engraftment)

Flow cytometry:

- stain, red cell lysis: 200,000 leukocytes/sample
- 50,000 events/measure (except diagnosis)

Standard panel: a standardized panel (antibodies/clone, combination, methods) should be used (see below).

- The panel was established by PD Dr. Griesinger, Göttingen, PD Dr. Dworzak, Vienna; PD Dr. Hrusak, Prague, Dr. Reinhardt, Muenster for the MRD-study of the AML-BFM 98 protocol.
- Analysis in Muenster is free of charge.

If a laboratory is not able to perform the upper mentioned analysis, leukemic blasts/nucleated cells should be isolated by Ficoll-gradient and frozen in liquid nitrogen. By PCR a sensitivity of \(10^{-2}\) to \(10^{-3}\) is possible, unfortunately only in 15% to 30% of the patients a molecular marker could be identified.

Send frozen samples (on dry ice) to Dr. Reinhardt, Pediatric Hematology/Oncology, University Muenster.

Appendix F Minimal Residual Disease


31 July 01/52
**Proposal of a standard panel**

**Diagnosis:**

<table>
<thead>
<tr>
<th>FITC PE</th>
<th>PerCP</th>
<th>MRD antibodies</th>
<th>antibodies</th>
<th>clone</th>
<th>order</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syto-16 CD7 CD45</td>
<td></td>
<td>Viability/Reference absolute count</td>
<td>Syto-16 Molecular Probes color</td>
<td>S-7578</td>
<td></td>
</tr>
<tr>
<td>CD13 CD33 CD34</td>
<td></td>
<td>Myeloid precursor</td>
<td>CD13 Dako FITC WM47 F 0831</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD15 CD117 CD34</td>
<td></td>
<td>Myeloid precursor/asynchronous antigen expression</td>
<td>CD15 BD FITC MMA 347423</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34 CD56 CD33</td>
<td></td>
<td>Leukemic immunophenotype</td>
<td>CD34 IT FITC 581 IM1870</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34 CD7 CD33</td>
<td></td>
<td>Leukemic immunophenotype</td>
<td>CD2 IT FITC 39C1.5 IM0442</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34 CD19 CD33</td>
<td></td>
<td>Leukemic immunophenotype</td>
<td>CD7 IT PE 8H8.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD2 CD13 CD34</td>
<td></td>
<td>Leukemic immunophenotype</td>
<td>CD33 BD PE P67.6 347787</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD38 HLA-DR CD34</td>
<td></td>
<td>Proliferation/HLA-DR/best antigen combination according to diagnosis</td>
<td>CD117 IT PE 95C3 IM1360</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD15 CD11c CD34</td>
<td></td>
<td>Granulocyte/monocyte/precursor</td>
<td>CD56 BD PE NCAM 16.2 340363</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8 CD3 CD4</td>
<td></td>
<td>T-lymphocyte</td>
<td>CD19 BD PE 4G7 329209</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD36 GlyA Apo 2.7</td>
<td></td>
<td>Red cell/erythroblast/late apoptosis</td>
<td>CD13 BD PE L138 347837</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD61 CD33 CD34</td>
<td></td>
<td>Megakaryocytic leukemia</td>
<td>CD45 BD PerCP 2D1 347464</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD34 IT PerCP 581 IM2648</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD33 Medac TC CD33-4D3 L11211</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD33 IT PerCy5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Bold red: fixed three-color panel (core)**

black: additional combinations at diagnosis; free
CD34 negative leukemia: Substitution of CD34 by HLA-DR

If immunophenotyping according to this proposal is not possible, please send frozen BM-sample to Muenster (Dr. D. Reinhardt, Hematological laboratory, Pediatric Hematology/Oncology, Albert-Schweitzer-Str. 33, D-48129 Muenster, Germany)
### Immunophenotyping „Minimal residual disease“

<table>
<thead>
<tr>
<th>Hospital:</th>
<th>Patient: (label)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Address:</td>
<td>Name:</td>
</tr>
<tr>
<td></td>
<td>Prename:</td>
</tr>
<tr>
<td></td>
<td>Birth-date:</td>
</tr>
<tr>
<td>Telephone:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Relapse:** [ ] no [ ] yes
- **Refractory:** [ ] no [ ] yes
- **Leukemia:** [ ] yes
- **Secondary malignancy:** [ ] no [ ] yes

**Immunological data:** .................................................................(immunophenotype, if available)

**Last treatment block:** .................

- **Start:** ....../......./....... till ....../......./........

- **Neupogen (G-CSF):** [ ] no [ ] yes, ....../......./....... till ....../......./........

**Material:**

- [ ] Bone marrow (heparinized)
- [ ] Ficoll isolated nucleated cells

**BMA:** ....................... (date)

- **hemoglobin:** ........ g/dl
- **WBC:** ............x10⁹/l
- **Platelets:** ..........x10⁹/l
- **BM-blasts:** ........... %

**Send to:**
Dr. D. Reinhardt, Hematological laboratory, Pediatric Hematology/Oncology, Albert-Schweitzer-Str. 33, D-48129 Muenster, Germany
Appendix G  Pharmacokinetics of liposomal Daunorubicin

Blood sampling
Day 1: End of infusion
   2 - 6 h after the end of infusion
   12 - 17 h after the end of infusion
   20 - 27 h after the end of infusion before the start of second infusion

Day 2: End of infusion
   20 - 27 h after the end of infusion before the start of second infusion

Day 3: End of infusion
   2 - 6 h after the end of infusion

Day 5: 20 - 30 h after the end of infusion
   30 - 40 h after the end of infusion

Preparation and storage
After centrifugation of about 400 µl blood (heparin or EDTA) transfer 200,0 µl plasma in special tubes filled with glycerol provided by the lab of Prof. Boos. Mix the plasma with the glycerol until no phase separation is visible and store at < -18°C until shipping.

Note infusion- and blood sampling times in the sampling-procedures (documentation of the times is more important than to keep the exact schedule)
The preparation and the blood sampling are described in the sampling-procedures.

Dispatch
Ship samples on dry ice to:

Prof. Dr. Joachim Boos
Klinik und Poliklinik für Kinderheilkunde
- Pädiatrische Hämatologie /Onkologie -
Albert-Schweitzer-Straße 33
48129 Münster
DaunoXome Sampling-Procedures
Protocol Relapsed AML 2001/01

Name: .................................................................

born: .......... Weight: .......... kg Height: .......... cm BSA: .......... m²

Dose absolute: .......... mg DaunoXome

<table>
<thead>
<tr>
<th>DaunoXome-infusion</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td></td>
<td></td>
<td></td>
<td>09.08.00</td>
</tr>
<tr>
<td>Start of infusion (time)</td>
<td>:</td>
<td>:</td>
<td>:</td>
<td>09:45</td>
</tr>
<tr>
<td>End of infusion (time)</td>
<td>:</td>
<td>:</td>
<td>:</td>
<td>10:50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Day of the protocol</th>
<th>Recommended sampling time</th>
<th>time</th>
<th>comments/sample number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>After the end of infusion</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2-6h after end of infusion</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12-17h after infusion</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Before start of 2 infusion</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>After the end of infusion</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Before start of 2 infusion</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>After the end of infusion</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2-6h after end of infusion</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>20-30h after infusion</td>
<td>:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>30-40h after infusion</td>
<td>:</td>
<td></td>
</tr>
</tbody>
</table>

- After centrifugation of about 400 µl blood (heparin or EDTA) transfer 200 µl plasma in special tubes filled with glycerol provided by the lab of Prof. Boos. Mix the plasma with the glycerol until no phase separation is visible and store at < - 18°C until shipping.
- Note infusion- and blood sampling times in the sampling protocol (documentation of the time is more important than to keep the exact schedule)
- Ship samples on dry ice

Hospital identifier

For correspondence refer to:

Phone:
## Appendix H  Group representatives for clinical and data management

<table>
<thead>
<tr>
<th>Clinical Management</th>
<th>Data Management</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AIEOP</strong></td>
<td></td>
</tr>
<tr>
<td>Carmelo Rizzari</td>
<td>Roberto Rondelli</td>
</tr>
<tr>
<td>e-mail</td>
<td></td>
</tr>
<tr>
<td><a href="mailto:carmelo.rizzari@tiscalinet.it">carmelo.rizzari@tiscalinet.it</a></td>
<td><a href="mailto:rondelli@med.unibo.it">rondelli@med.unibo.it</a></td>
</tr>
<tr>
<td>phone</td>
<td></td>
</tr>
<tr>
<td>+39 – 39 233 3513</td>
<td>+39 – 51 636 4667</td>
</tr>
<tr>
<td>fax</td>
<td></td>
</tr>
<tr>
<td>+39 – 39 230 1646</td>
<td>+39 – 51 34 5759</td>
</tr>
<tr>
<td><strong>AML-BFM-G</strong></td>
<td></td>
</tr>
<tr>
<td>Ursula Creutzig</td>
<td>J.E. Müller</td>
</tr>
<tr>
<td>e-mail</td>
<td></td>
</tr>
<tr>
<td><a href="mailto:Ucreutzig@aol.com">Ucreutzig@aol.com</a></td>
<td><a href="mailto:mullerj@uni-muenster.de">mullerj@uni-muenster.de</a></td>
</tr>
<tr>
<td>phone</td>
<td></td>
</tr>
<tr>
<td>+49 – 251 835 6486</td>
<td>+49 – 251 835 6487</td>
</tr>
<tr>
<td>fax</td>
<td></td>
</tr>
<tr>
<td>+49 – 251 835 6489</td>
<td>+49 – 251 835 6489</td>
</tr>
<tr>
<td><strong>BFM-A:</strong></td>
<td></td>
</tr>
<tr>
<td>Helmut Gadner</td>
<td>Nora Muehlegger</td>
</tr>
<tr>
<td>e-mail</td>
<td></td>
</tr>
<tr>
<td><a href="mailto:Gadner@ccri.univie.ac.at">Gadner@ccri.univie.ac.at</a></td>
<td><a href="mailto:Muehlegger@ccri.univie.ac.at">Muehlegger@ccri.univie.ac.at</a></td>
</tr>
<tr>
<td>phone</td>
<td></td>
</tr>
<tr>
<td>+43 – 1 4017 0250</td>
<td>+43 – 1 4017 0478</td>
</tr>
<tr>
<td>fax</td>
<td></td>
</tr>
<tr>
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Appendix I

Patient data and follow-up form

Attached separately as 1 file (Intern-Rel-01-Registrat-B)!
(4 pages)

Please replace this page by the appropriate print-outs!
Appendix J  
NCI-Common Toxicity Criteria (modified by SIOP)

Attached separately as 1 file (DOK_Intern-Toxicity)
(1 page)

Please replace this page by the appropriate print-out!
Appendix K

Therapy checklist/worksheets

Attached separately as 5 files (DOK_Intern-FLAG-1, DOK_Intern-FLAG-2, DOK_Intern-FLAG-DNX1, DOK_Intern-Consolid, DOK_Intern-Consolid-VP)
(5 pages)

Please replace this page by the appropriate print-outs!
Appendix L

Flowchart of the protocol

Attached separately as 1 file (protocolB.lbfmsg)
(1 page)

Please replace this page by the appropriate print-out!